Journal of the American Association for Laboratory Animal Science
Copyright 2021
by the American Association for Laboratory Animal Science

Vol 60, No 6
November 2021
Pages 692–699

Evaluation of Pain and Distress and Therapeutic Interventions for Rectal Prolapse in Mice to Reduce Early Study Removal

Cara M Mitchell,[*] Gregory W Salyards,[§] Betty R Theriault, George P Langan, and Kerith R Luchins

Rectal prolapse (RP) is a common clinical condition in mice, that does not have a recognized or documented standard of care. At our institution, an average of 240 mice develop RP each year. Our practice has been to recommend euthanasia upon identifying a RP based on its appearance as a painful or distressful condition. This study aimed to assess treatment options that would maintain the RP mucosa and allow mice to reach their study endpoint, and to evaluate the perception of this condition as a painful or distressful event. This study used 120 mice with spontaneous RP, concurrently assigned to ongoing research protocols. Mice were randomly assigned to 1 of 3 treatment groups: petroleum jelly, lidocaine jelly, or no treatment. Fecal samples were collected for pathogen testing, and all mice received an initial base score, followed by weekly blind scores. Upon euthanasia, RP tissue was collected for histopathology. Of the 120 mice identified with RP, 47 mice were breeders; 28% successfully produced 22 additional litters after developing RP. Seventy-three were nonbreeders, with 92% reaching their research study endpoint. No statistically significant differences were detected between the 3 treatment groups based on gross mucosal health, pain and distress, or histopathology. In this study, none of the mice in any group were euthanized based on the RP endpoint scoring criteria. These findings demonstrate that treatment is unnecessary for RP, and mice with RP did not show signs of pain or distress. In adherence to the 3Rs, this study supports animal number reduction and clinical refinement, allowing mice with RPs to reach their intended research study endpoints or produce additional litters.

Abbreviations: ARC, Animal Resources Center; BS, base score; BCS, body condition score; LJ, lidocaine jelly; CT, control; F, female; GMH, gross mucosal health; IBD, inflammatory bowel disease; M, male; PAD, pain and distress; PJ, petroleum jelly; PP, parent protocol; U of C, University of Chicago; RP, rectal prolapse(s); WS, weekly score

DOI: 10.30802/AALAS-JAALAS-21-000038

Rectal prolapse (RP) is a common clinical condition in laboratory mice. Currently, the laboratory animal medicine community has no generally accepted or documents standard of care for managing mice with RP. Institutional practices vary and typically consist of no treatment, applying lubrication to the RP mucosa, dextrose to reduce RP, surgical correction, or euthanasia.[3,11,34]

In larger animals, such as sheep, horses, nonhuman primates, cats, and dogs, surgical correction of a RP is often successful.[1,17,39] In mild or acute cases, medical management with warm saline lavage, water-soluble lubrication, or hypertonic sugar solution can be used to aid in complete or partial reduction of the RP.[1,39] Surgical correction of RP has been described in mice; however, complications such as trauma and lack of defecation, risks associated with anesthesia, and providing postoperative care must be considered.[34] A simpler and less costly option is generally to euthanize and replace the mouse.

In humans with RP, studies show that surgery is not always the best option depending on age or pathogenicity.[4,8,28,29] Conservative methods are often considered the first line of treatment, especially in children.[4,10,25] In humans, a prolapsed rectum is reported to be a benign, nonpainful condition and rarely is an emergency.[8,14,28,37] Often, people with RP describe the feeling of something falling out of their rectum or akin to sitting on a ball.[14,28,37] In these studies, most people elect to have surgical correction due to the effects of a persistent RP on quality of life; such as incontinence, constipation, mucous discharge, and tenesmus.[14,28,37]

In mice, causes for RP include stress, pregnancy and parturition, age, genetics, study manipulations, or infectious pathogens.[2,12,13,23,27,30,40] Mice of some strains or genetic backgrounds are more likely to develop RP, such as transgenic knockout mice deficient in urokinase type plasminogen activator, nonmuscle myosin II cKO, and IL10 KO strains.[26,32,40] Study manipulations and inducible models of colitis or gastrointestinal neoplasia are also associated with a higher likelihood of developing a RP.[19,32] Infectious pathogens such as *Helicobacter* spp., *Citrobacter rodentium,* and the murine pinworms, *Syphacia obvelata* and *Aspiculuris tetraptera,* are also commonly associated with RP due to inflammation of the lower bowel.[2,12,13,38] All of these factors along with the short rectum of mice (1 to 2 mm) predisposes them to RP.[2]

At the University of Chicago (U of C), Animal Resources Center (ARC), the standard recommendation upon identification of the RP is euthanasia because the condition is thought to be painful or distressing. Depending on the study and whether a RP is anticipated, the RP is described in the IACUC protocol along with scientific justification for maintaining the mouse

Received: 30 Mar 2021. Revision requested: 11 May 2021. Accepted: 06 Jul 2021.
[1]*Animal Resources Center and Department of Surgery, University of Chicago, Chicago, Illinois*
[*]*Corresponding author. Email:maecara555@yahoo.com*
[§]*Current affiliation: Division of Veterinary Resources Office of Research Services, National Institutes of Health, Bethesda, Maryland*

with a RP. When a RP is to be maintained, the early endpoint criteria require euthanasia if the mucosa becomes necrotic, self-mutilation occurs, or the RP is > 5 mm. At our institution, on average 240 mice are euthanized each year due to RP. With the knowledge that a RP is a benign process in humans, the objective of the current study was to clinically assess spontaneous RP in mice currently assigned to ongoing research protocols for pain and distress and to compare treatment options intended to maintain healthy RP mucosa. Researchers are responsible for treating their mice at U of C; therefore, the 2 topical treatments chosen for this study were practical, inexpensive, and straightforward to ensure compliance. The ultimate aim of the study was to refine the standard treatment in mice with RP to eliminate the need for early study euthanasia and allow mice to reach their study endpoint. We hypothesized that both treatments would maintain the RP mucosa better than no treatment, allowing mice to reach their study endpoint and allow us to assess the perception of this condition as painful or distressful.

Materials and Methods

Husbandry and Animal Care. All mice were housed on IVC racks (Allentown Jag 75 Micro-VENT Environmental System IVC racks, Allentown, Allentown, NJ). Most mice were group housed in Allentown Jag 75 Micro-Barrier (Allentown, Allentown, NJ) solid-bottom polycarbonate individually ventilated cages (19.69 × 30.48 × 16.51 cm). The majority of mice were housed on 1/4-inch corncob bedding (Teklad 7097, Envigo, Indianapolis, IN), provided ad lib water either by reverse osmosis through an automatic watering system (Avidity Science [previously Edstrom Industries], Waterford, WI) or acidified tap water provided in bottles, and fed an irradiated diet (Teklad 2918, Envigo, Indianapolis, IN). PIs have the option to house mice on either cellulose bedding (Teklad 7089, Envigo, Indianapolis, IN) or shredded pine shavings (Item 326.2, NEPCO, Northeastern Products, Warrensburg, NY), however, this is not commonly done. Mice were provided shredded paper (Bedrnest [The Andersons INC, Maumee, OH] or Enviro-dri [Shepherd Specialty Papers, Watertown, TN]) for enrichment. All cages, bedding, and enrichment were autoclaved prior to use. Animal cages were changed every 14 d in a Class II Type A2 Biosafety Cabinet (NuAire, Plymouth, MN). Animal rooms were maintained on a 12:12-h light:dark cycle with humidity ranging from 30% to 70% and temperatures ranging from 68 to 76 °F (20-24.4°C). Mice were checked daily by the animal care staff to assure good health and that appropriate food, water, and cage conditions were present. Excluded agents identified by exhaust air dust testing via PCR, were Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse parvoviruses, reovirus, epizootic diarrhea of infant mice, mouse encephalomyelitis virus, ectromelia virus, lymphocytic choriomeningitis virus, murine adenovirus, murine cytomegalovirus, K virus, polyoma virus, mouse thymic virus, hantavirus, lactate dehydrogenase-elevating virus, *Filobacterium rodentium*, *Mycoplasma pulmonis, Salmonella* spp., *Citrobacter rodentium, Clostridium piliforme, Streptobacillus moniliformis, Corynebacterium kutscheri,* and endo- and ectoparasites such as *Hymenolepis* spp., *Giardia muris, Encephalitozoon cuniculi*, *Myobia musculi, Myocoptes musculinus, Radfordia affinis, Psoregates simplex, Syphacia* spp., and *Aspiculuris tetraptera*. Mouse norovirus, *Rodentibacter pneumotropicus* and *R. heylii* (previously *Pasteurella pneumotropica*), *Helicobacter* spp., and segmented filamentous bacteria were endemic in the vivaria except in a few designated rooms. These rooms represent approximately 2,000 cages, or 10% of our total census. No mice from these rooms were used for this study. All procedures and housing followed the *Guide for the Care and Use of Laboratory Animals,* 8th edition.[16] The Animal Care Program at the U of C is AAALAC-accredited, and all animal work was approved by the U of C's IACUC.

Animals. During a 6-mo period (07/01/2019-01/22/2020), 120 mice with RP were identified by the animal care staff within 3 different animal barrier facilities at the U of C, with no limitations imposed due to age, sex, strain, or genetic background. The combined daily census of the 3 facilities was approximately 20,000 cages of mice. This study used mice that were actively assigned to ongoing research protocols (parent protocols; PP) and were found with a spontaneous RP. This design was intended to represent the actual population of mice at risk for developing RP at biomedical research institutions. All principal investigators were notified prior to the treatment of RP for approval of use in this study. Housing was based on the PP and was not changed for this study, so group housed mice remained together. Age, sex, strain, previous experimental manipulations, and IACUC PP endpoints were obtained for each mouse. Mice with any preexisting non-research related comorbidities were not included in this study.

Experimental Design. Once a rectal prolapse was identified, the mouse was assigned a rectal prolapse base score (BS) within 2 d. BS included the following criteria: measurement (mm) of the rectal prolapse (protrusion of the rectal tissue: distance from the base of the anus to most distal tip of the RP), gross mucosal health (GMH, Table 1), pain and distress (PAD, Table 2)[3] and body condition score (BCS, 5-point scoring system).[35] Once a week during treatment, the RP was measured (mm) using the same digital calipers (CG-1162-V-50, 150mm [6″], Chemglass Life Sciences, Vineland, NJ) by the same veterinarian. The digital calipers were calibrated each day prior to use for RP measurements. Each mouse was randomly assigned to 1 of 3 treatment groups: petroleum jelly (PJ) (Curad Petroleum Jelly, Mundelein, IL), lidocaine jelly (LJ) (GLYDO Lidocaine HCL Jelly USP 2%, Schaumburg, IL) or no treatment (control, CT) using a random number generator.[15] Treatment was administered to the RP mucosa 3 times a week (Monday, Wednesday, and Friday). Approximately 0.05 mL or less (a thin coat) of LJ (1mg) or PJ was applied to the RP using a cotton tip applicator. This was done by placing the mouse on top of the wire bars and gently lifting up the tail. The mouse remained on the wire bars for

Table 1. Gross Mucosal Health of Rectal Prolapse

Score	Observation
0	Pink to red healthy mucosa.
1	Inflamed/edematous, dry, pinpoint blood spots or defects in the mucosa (indicating possible erosion, excoriation, or ulceration).
2	Necrotic mucosa, self-trauma, or overt hemorrhage.

Table 2. Assessment of Pain and Distress in Mice[3]

Score	Observation
1 No indication of pain/distress	Normal; well-groomed, alert, active, good condition, asleep, or calm.
2 Mild or anticipated pain/distress	Not well-groomed, awkward gait, slightly hunched.
3 Moderate pain/distress	Rough hair coat, squinted eyes, moves slowly, moderately hunched, depressed, lethargic.
4 Severe pain/distress	Very rough hair coat, severely hunched, nonresponsive, dyspnea, dehydration.

approximately 1 min. If bedding became stuck on the RP due to the treatment application, it was not removed, as removal often causes bleeding. Furthermore, the bedding falls off on its own or the mouse removes it within a couple of hours.

Every 7 days on nontreatment days, each mouse received a weekly score (WS) based on the GMH, PAD, and BCS. These scores were assigned by a pool of 5 veterinarians and veterinary technicians (veterinary staff) trained for this study, and blinded to previous scores and treatment groups. To ensure interrater agreement on the WS, each mouse was scored by 2 individuals for the first 2 mo. Mice continued to receive WS and treatment until the PP endpoint, or for a maximum of 3 mo (RP study endpoint), or until meeting euthanasia criteria (GMH score of a 2 (Table 1), PAD score of a 3 (Table 2),[3] or BCS < 2/5). If at any point a mouse met one of the criteria for euthanasia, 2 additional members of the veterinary staff (one veterinarian and one veterinary technician) would provide individual scoring for the mouse. The consensus of at least 2 of the individuals would determine the outcome for this mouse. If new litters were present in the cage at the time of treatment or during WS, the RP was not evaluated or treated for 3 d so as not to disturb the new pups.

Fecal PCR. At the BS, each animal enrolled in this study had a fecal sample collected directly from the rectum or taken from the cage to test for *Helicobacter* spp. and pinworms (*Syphacia* spp. and *Aspiculuris tetraptera)* prior to starting treatment. These pathogens were tested at the animal level because they are documented as the most common cause of RP in mice.[2,13] Samples were submitted to IDEXX BioAnalytics (Discovery Ridge Research Park, Columbia, MO). No other infectious pathogens were directly tested in the study mice as other common pathogens that cause RP were monitored quarterly by exhaust air dust testing via PCR.[21]

Histopathology. When available based on research needs, prolapsed rectal mucosa and a small section of the colon were collected at the time of euthanasia, placed in formalin (10% buffer solution), and submitted to IDEXX BioAnalytics (Discovery Ridge Research Park, Columbia, MO) for assessment. Histopathology allows an objective assessment of how the 2 treatments (PJ and LJ) maintained mucosal health compared with the CT. A RP mucosa scoring table was created and evaluated by a board-certified veterinary pathologist blind to the history of the submitted samples. Eight mucosal characteristics on the everted mucosa were identified and scored (Table 3).

Statistical Analysis and Sample Size Determination. Statistical analyses were performed using Stata, v16.1 (StataCorp, College Station, TX). The data was expressed as mean with standard deviation and P<=0.05 was regarded as statistically significant. The chosen primary endpoint was time from treatment randomization to euthanasia. Times to euthanasia was estimated by the Kaplan-Meier method[18] and compared between the treatment arms by a log-rank test. Analyses were performed using 2 different assumptions, that is, a survival rate in the control arm of 10% at 2 wk compared with 50% in either treated arm, or 25% in the control group compared with 70% in either treatment arm, which corresponded to cause-specific hazard ratios of 0.30 and 0.26, respectively, $n = 40$ mice per group provided an 85% statistical power. This calculation also conservatively assumed a 50% loss rate due to deaths from other causes.

GMH, PAD, and BCS, were secondary endpoints. Interrater agreement in the scoring of GMH, PAD, and BCS was assessed by calculation of κ statistics. For all 3 of these ordinal variables, only 2 levels of outcomes were actually observed. Therefore mixed-effects logistic regression models were fit to analyze the longitudinal changes over time in these outcomes with treatment, time, and treatment-by-time interaction terms as fixed effects and animal as a random effect to allow for correlation within animal.[7] In addition, because all BCS scores in the lidocaine treatment arm remained constant over time, the final BCS score recorded for each animal was compared across the 3 treatment groups using the Fisher exact test.

Changes in rectal prolapse size were analyzed by fitting ordinary mixed-effects regression models with the same fixed and random effects as those used in the logistic models. The main effects of interest here were the treatment-by-time interaction terms, which compared the rates of change in rectal size (slopes) across the groups. Finally, histology parameters obtained upon euthanasia of the mice (hyperplasia, goblet cell loss, erosion, crypt abscess, inflammation, crypt irregularity, and erosion type) were analyzed by Kruskal–Wallis nonparametric tests. The presence or absence of bacteria was compared between groups using the Fisher exact test.

Results

Animals. One hundred and twenty mice, 66 male (M) and 54 female (F), were identified with spontaneous RP; 73 (61%) were nonbreeding, and 47 (39%) were breeders. Mice were identified from the protocols of 16 different principal investigator. Parent protocol research studies included immunology, endocrinology, and oncology. RP was most commonly found in the strains Rag-deficient (n=22), Foxp3^{DTR} (n=24), and IL10 KO (n=27). Average age of onset of RP was 112 d old (3.7 mo) with the youngest age of 46 d (1.5 mo) and the oldest age of 295 d old (9.8 mo). None of

Table 3. Prolapsed Rectal Mucosa Histopathology Scoring

Mucosa characteristics	0	1-mild	2-moderate	3-marked
Hyperplasia	neg	< 20% (increase cell numbers in longitudinal crypts)	20% to 50% (increase cell numbers in longitudinal crypts, ± mitoses in middle/upper third of crypt distant from base)	> 50% (increase cell numbers in longitudinal crypts, ± mitoses in upper third of crypt distant from base)
Goblet cell loss	neg	< 20%	20% to 50%	> 50%
Erosion %	neg	< 20%	20% to 50%	> 50%
Cryptitis	neg	1 or 2	3–9 total	> 9 total
Inflammation	neg	mild density	moderate density	marked density
Irregular crypts	neg	< 50%	> 50%	> 50% AND/OR scattered herniation of crypts beneath muscularis mucosae
Bacteria	neg	pos	N/A	N/A
Erosion character	neg	loss of mucosa width of single crypt	loss mucosa in 2–4 contiguous crypts	loss mucosa in 5 or more contiguous crypts

Table 4. 13 Breeder mice are in order from smallest RP to largest, with the number of litters produced and the treatment group. Of the 13 breeders, 6 were in the PJ group, 4 in the LJ group, and 3 in the CT group.

Mean RP Size (mm)	Sex	# of litters	Treatment
1.4	F	2	CT
1.5	F	3	CT
1.5	F	2	LJ
1.8	F	1	PJ
2.0	F	1	LJ
2.2	F	1	PJ
2.7	M	1	PJ
2.7	F	2	LJ
3.4	F	1	LJ
3.7	M	1	PJ
4.0	M	2	PJ
4.5	M	3	PJ
4.6	F	2	CT

Treatment: CT, Control; LJ, Lidocaine jelly; PJ, Petroleum jelly
Sex: M, Male; F, Female

the 120 mice in any group (LJ, PJ, or CT) were euthanized based on the RP euthanasia criteria. As a result, a statistical analysis of this endpoint was not conducted.

Breeding Mice. Of the 47 breeders (19 M and 28 F), 24 (51%, 14 M and 10 F) were euthanized for not breeding within 4 wk as required for the PP endpoint. Among the remaining 23 mice, 13 (57%, 4 M and 9 F) continued to breed after presenting with a RP, producing a total of 22 additional litters (Table 4). Of the 13 that continued to breed, 5 reached the 3 mo RP study endpoint, 5 reached the PP endpoints, and 3 were euthanized due to factors unrelated to the RP. For the remaining 10 (10/23), 3 RP resolved and 7 were euthanized due to factors unrelated to the RP.

Non-Breeding Mice. Of the 73 nonbreeding mice (61%, 47 M and 26 F), 6 were euthanized or found dead for health reasons not related to PP or RP. Fifty-three (72%) mice met the PP endpoint, with 30 (56%) of those mice reaching both the PP endpoint and the RP study endpoint of 3 mo.

Rectal Prolapse Size The 120 mice provided a total of 1,118 measured observations (mean 9.3, min 2, max 18 per mouse). At the BS, the mean size of the RP was 2.8 mm (min 0.9 mm and max 5.2 mm); at euthanasia, the mean size of the RP was 3.2 mm (min 0.8 and max 7.1, Figure 1). Each group, PJ, LJ, and CT, had an increase in RP size over time, with a linear regression growth of 0.27, 0.14, and 0.26 mm per month, respectively. No statistically significant difference ($P = 0.75$) was detected in the size of the RP over time between the PJ and the CT group. However, the rate of RP growth in the LJ treatment group was about half that of the CT group (0.14 compared with 0.26 mm per month), which was a statistically significant difference ($P = 0.001$).

Blind Weekly Scoring (WS). In the first 2 mo of the study, each mouse received scores from 2 different individuals. This was done to determine interrater agreement for the RP scoring criteria of GMH, PAD, and BCS. GMH showed an 88% agreement (expected agreement by chance, 76%), PAD a 91% agreement (expected by chance, 74%), and BCS 98% agreement (expected agreement by chance, 90%).

For all treatment groups, the GMH scores were 0 or 1 (Figure 2), with no mice reaching a score of 2. The PAD scores remained at 1 or 2, with no mice reaching a score of 3. GMH and PAD showed no statistically significant difference between the 2 treatments (PJ, LJ) as compared with the CT group. However, in all 3 groups, the odds of GMH being at a score of 1 rather than level 0 increased over time (odds ratio = 1.06 per month, $P = 0.011$). Similarly, the odds of PAD being at a score of 2 rather than 1 also increased over time (odds ratio = 1.51 per month, $P < 0.001$). For the BCS, the final scores of all mice in the PJ and CT groups were between 2/5 and 3/5 , whereas for the LJ group, all final BCS were with 3/5, which differed significantly from the CT group (Fisher exact $P = 0.012$).

Figure 1. Largest rectal prolapse 7.1 mm. Nonbreeding male (Rag deficient, age of onset 137 d) in the CT group, which met both its parent protocol research study endpoint and our rectal prolapse 3-mo endpoint, with a GMH of 1, PAD of 2, and BCS of 2/5.

Fecal PCR. Of the 120 mice, 115 (96%) tested positive for *Helicobacter spp: H. mastomyrinus, ganmani, hapaticus, typhlonium, rodentium* and *bilis*. *H. mastomyrinus* was the most common (78%) species found both as a sole agent and in coinfections. (Figure 3). Five mice tested negative for all *Helicobacter* spp. All mice tested negative for pinworms, *Syphacia* spp. and *Aspiculuris tetraptera.*

Histopathology. From the 120 mice, 108 RP samples were collected (90%, 62 M and 46 F, 57 breeders and 51 nonbreeders) and sent for histopathology. Samples were collected at euthanasia. Twelve mice were lost to follow-up and therefore their RP were not collected for histopathology. No statistically significant differences were found between RP mucosal scores of the 3 groups: hyperplasia ($P = 0.12$), goblet cell loss ($P = 0.75$), erosion% ($P = 0.32$), cystitis ($P = 0.48$), inflammation ($P = 0.63$), irregular crypts ($P = 0.88$), bacteria ($P = 0.70$), erosion type ($P = 0.85$) (Table 5).

Discussion

Spontaneous RP is a common clinical condition that affects both male and female mice. At the U of C, we find approximately 240 mice with a RP per year. Before this study, our standard practice was to euthanize any mouse with a RP unless scientifically justified extension was approved in the IACUC protocol. Based on the current study, we conclude that no treatment is necessary to maintain the RP GMH, as no significant difference was found between the 2 treatment groups as compared with

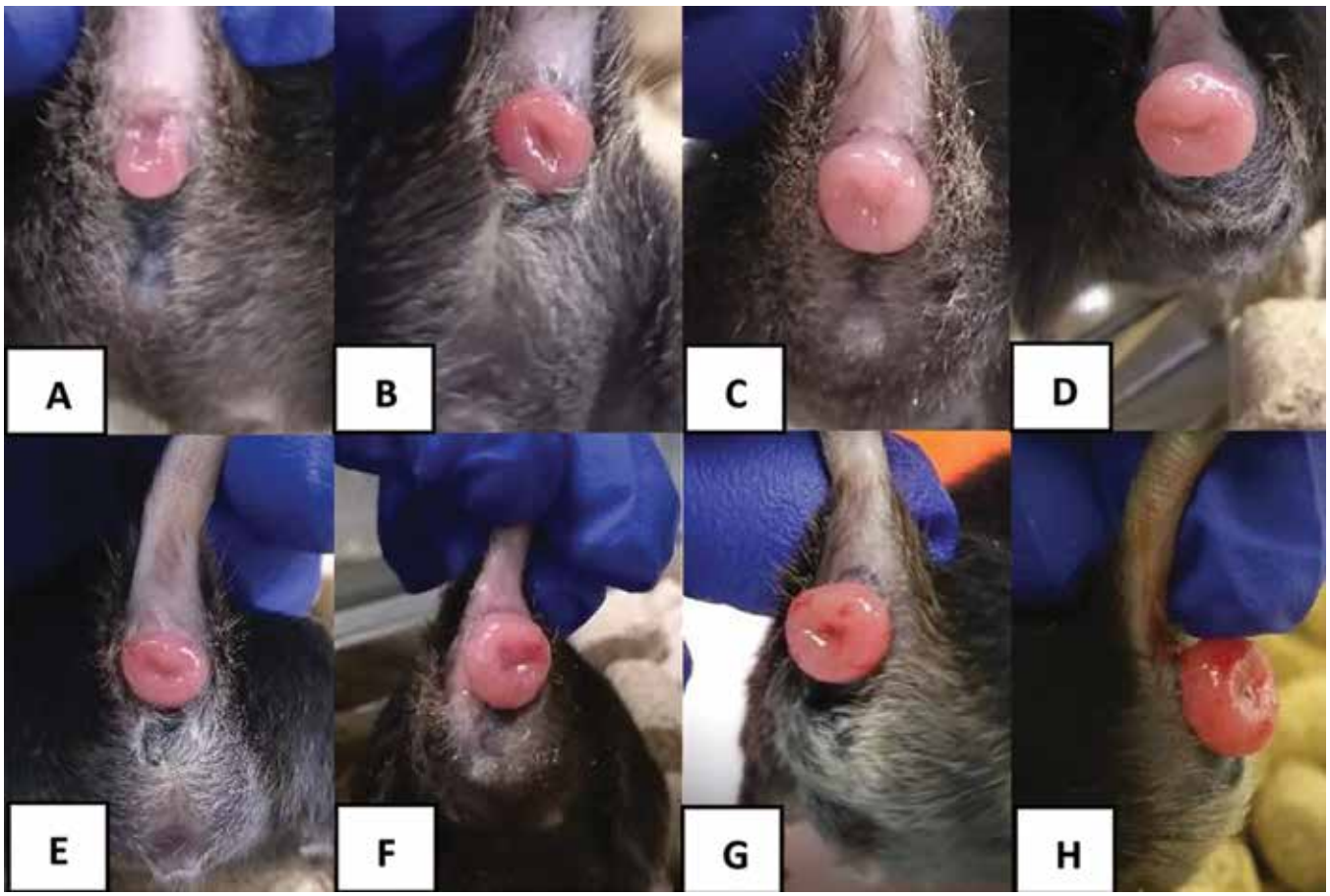

Figure 2. (A through D) Examples of a gross mucosal health score of 0. A) IL10 KO female breeder, onset 55 d,[a] 64 d on PJ. B: Irf4^{fl} male nonbreeder, onset 74 d,[a] 23 d on PJ. C: IL10 KO female nonbreeder, onset 105 d,[a] 43 d on LJ. D) Rag deficient male nonbreeder, onset 130 d,[a] 90 d on CT. (E through H) Examples of a gross mucosal health score of 1. E) Foxp3^{DTR} male breeder, onset 68 d,[a] 64 d on PJ. F) TCR female nonbreeder, onset 71 d,[a] 97 d on LJ. G) IL10 KO male breeder, onset 110 d,[a] 42 d on LJ. H) OT-1 male nonbreeder, onset 160 d,[a] onset 64 d on CT. [a]age of mouse when RP presented.

the CT. As for PAD, no mice received a score above a 2, and a significant difference between the treatment groups was not detected. Moreover, none of the 120 mice evaluated and treated in this study met our RP euthanasia criteria.

In choosing the topical treatments to be used for the study, we considered practicality of use, ease of obtaining the product, and cost in order to encourage compliance by the research staff. While dextrose, and other osmotic solutions, may help reduce the RP size and the need for replacement mice, our study specifically evaluated maintaining the RP as presented and determining whether welfare concerns were associated with the RP. We chose petroleum jelly due to its ability to help retain moisture, as it has few to no side effects based on chemical properties[9,31] To manage the possibility of pain associated with a RP, we used lidocaine jelly (Glydo) to provide a temporary local analgesic.[6] Both of these products are reasonably priced and can be purchased in-house by our researchers. The potential of toxicity from lidocaine jelly was a consideration. The product's insert states that the oral LD_{50} in nonfasted rats is 459 mg/kg. The mice in the LJ group received approximately 0.05 mL (1 mg) or less 3 times a week, although we cannot state the exact amount that was applied to the RP (mean size 2.8 mm) or the dose that was received. However, we anticipated that the amount applied would not cause toxicity, and no animals were observed with CNS signs after application.

In developing this project, we did not anticipate that breeding mice with an RP would continue, especially not the females, expecting that the RP would interfere with copulation. However, our data show that mice with RPs can copulate and produce additional litters (Table 4). When identifying breeders with a RP, most PIs chose to euthanize after 4 wk due to unsuccessful breeding. Some of our mice were first time breeding pairs, while others were established breeders. Of the 47 breeders with RP, 24 were euthanized for not breeding within a 4 wk period. However, this failure to breed could have been due to the RP, pairing incompatibility, or other factors.

Previously at the U of C, if a RP was approved in a protocol, our guidelines stated that mice whose RP was > 5 mm would require euthanasia. Because this was an arbitrary number, we removed length as a euthanasia criterion and instead based euthanasia on GMH, PAD, and BCS. We wanted to determine whether RP size was a factor in maintaining mucosal health or causing pain and distress to provide evidence supporting this decision. Although the RP of the LJ group grew at a significantly slower rate (50% of the CT), the slower growth did not statistically affect the GMH or PAD scores. Moreover, the LJ group all had a BCS of 3/5. Because these mice were on different PP and were subjected to different and ongoing study manipulations, we cannot infer that the 3/5 BCS is related to the LJ application, as that conclusion would require additional further studies.

For GMH, all 120 mice had scores of 0 (pink to red healthy mucosa) and 1 (inflamed or edematous, dry, pinpoint blood spots or defects in the mucosa [erosion, excoriation, or ulceration]). None of the mice reached a score of 2 (Necrotic mucosa,

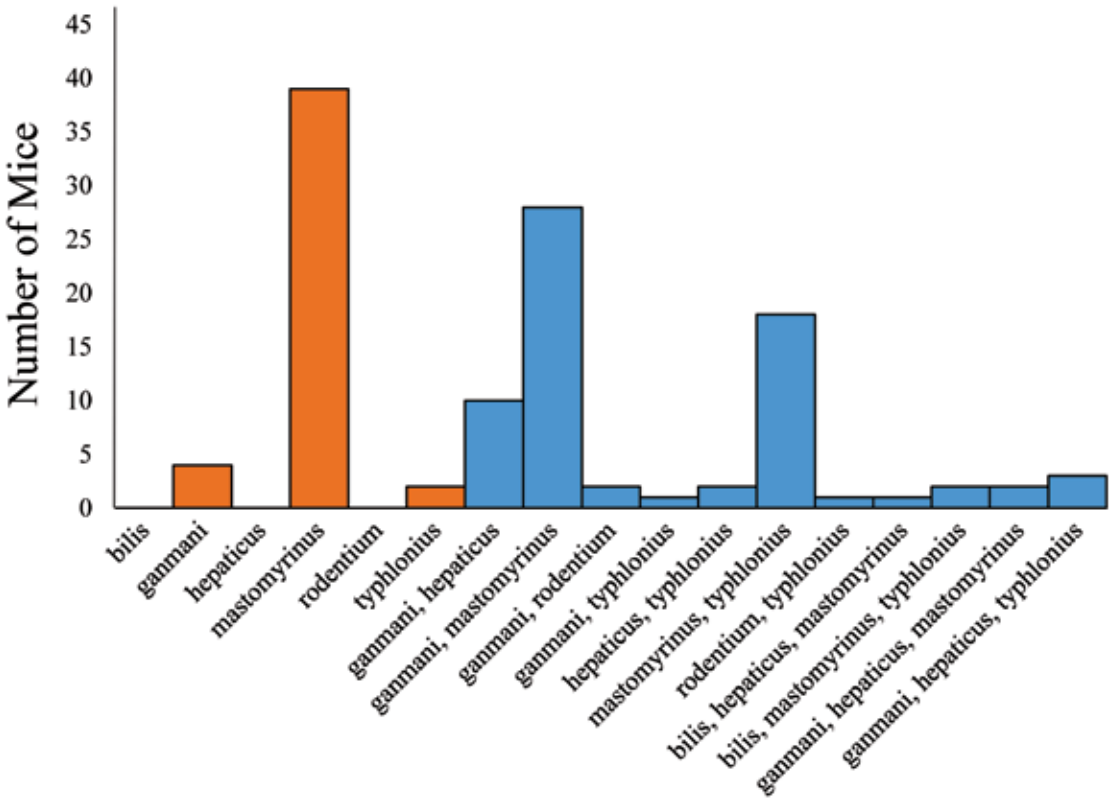

Figure 3. Fecal PCR results. This figure shows the number of mice that tested positive for the different *Helicobacter spp.* The 6 *Helicobacter spp.* reading from left to right, are the number of mice (orange bars) that tested positive for only one *Helicobacter spp.* The number of mice that tested positive for more than one species are represented by the blue bars.

self-trauma, or overt hemorrhage). For purposes of this study, we expected to identify a correlation in PAD or a decrease in the BCS with an increase in the GMH score. We initially anticipated that the RP in the CT group would become necrotic or have a higher GMH score than the PJ and LJ groups; however, this did not occur.

Our data revealed an odds ratio of > 1 for both GMH (score of 0 increasing to a score of 1 over time) and for PAD (score of 1 increasing to a score of 2 over time) in all 3 treatment groups. This implies that with increasing duration of the RP, the GMH and PAD scores would continue to rise. The increasing scores could portend potential welfare concerns. Because of this, mice with RP should be monitored regularly, as the current study had a duration of only 3 mo. Future studies should determine whether the RP can be humanely maintained past 3 mo.

Assessing pain and distress in prey species such as mice can be quite difficult. Reliability for evaluating pain and the manifestation of pain in mice can vary due to factors such as strain, severity and location of insult, type of pain, time of day, environmental factors, type of observation (video compared with cage side), and general bias from the observers.[3,20,24,33,36] Our decision to subjectively assess pain and distress using behavioral or attitudinal changes stemmed from our current practices. Our staff are trained to observe mice in their home cage, assessing activity level, grooming, hunched posture, and overall wellbeing. They are taught that pain and distress in an animal is often inferred from the absence of normal behaviors.[36] Because establishing a pain and distress baseline before the RP was not possible, our goal was to evaluate pain, distress, and clinical wellbeing overall at the time the RP presented and to monitor the chronic progression of this condition. The Grimace scale was not used because some studies show that this method is mostly used in acute pain studies concurrent with analgesics.[22] We did not measure physiologic parameters (e.g., heart rate, respiration, corticosterone), which are often used to indicate acute pain. We did not want to further stress the mice by blood collection or interfere with concurrent research.

Histopathology scoring was based on the characteristic scores commonly used to evaluate mouse IBD models. The mucosal characteristics with the highest scores (3, marked; > 50% involved) were hyperplasia, goblet cell loss, and irregular crypts. Grossly, no tissue necrosis was seen; however, microscopic cell necrosis, cell erosion, and goblet cell loss were observed. Studies assessing mouse models of IBD, pathogen related causes for RP, novel treatments, and age progression in mice report similar histopathologic findings.[3,23,27,30]

From the literature, we know that *Helicobacter* spp. can lead to RP due to IBD.[5,12,23] In our census of approximately 20,000 cages, *Helicobacter* spp. is considered endemic in our mouse barrier rooms based on historical testing, with only approximately 2,000 cages located in *Helicobacter* spp. free rooms. No RP were observed in our *Helicobacter. spp*. free rooms during the data collection time frame. Using the information we obtained from this study, only 0.5% (calculations not shown) of the entire mouse population infected with *Helicobacter spp.* developed a RP.

In this study we conclude that in our institution, no treatment is necessary to maintain mice with RP, but a few limitations should be considered. As with most species, maintaining an adequate contact time is difficult when using a topical treatment. Often, we must balance providing treatment and limiting stress. Given that these mice were on concurrent protocols, we wanted

Table 5. Histopathology data summary for each treatment (PJ, LJ, CT)

Variable	# Observations	Mean score	Std. dev	Min	Max
Petroleum Jelly					
Hyperplasia	38	3.00	0.00	3	3
Goblet cell loss	38	2.92	0.49	0	3
Erosion%	38	1.97	0.82	1	3
Cystitis	38	1.32	0.93	0	3
Inflammation	38	2.58	0.60	1	3
Irregular crypts	38	2.84	0.44	1	3
Bacteria	38	0.61	0.50	0	1
Erosion type	38	2.66	0.53	1	3
Lidocaine Jelly					
Hyperplasia	35	2.94	0.24	2	3
Goblet cell loss	35	2.97	0.17	2	3
Erosion%	35	2.14	0.81	1	3
Cystitis	35	1.43	1.04	0	3
Inflammation	35	2.43	0.70	1	3
Irregular crypts	35	2.86	0.36	2	3
Bacteria	35	0.60	0.50	0	1
Erosion type	35	2.60	0.55	1	3
Control					
Hyperplasia	35	3.00	0.00	3	3
Goblet cell loss	35	2.91	0.37	1	3
Erosion%	35	2.26	0.78	1	3
Cystitis	35	1.60	1.12	0	3
Inflammation	35	2.54	0.66	1	3
Irregular crypts	35	2.80	0.47	1	3
Bacteria	35	0.69	0.47	0	1
Erosion type	35	2.54	0.70	1	3

to avoid causing additional variables to the PP protocol. Another limitation of the study involves the bedding used. The majority of mice were housed on 1/4-inch corncob bedding. However, 4 mice (3%) were housed on either shredded pine shavings or cellulose bedding. For this study, we did not compare the GMH of the RP in relation to the different types of bedding. As noted earlier, we did not manually remove bedding that was stuck to the RP. Because of the limited number of mice housed on woodchips or paper bedding, we cannot comment on whether different types of bedding would have influenced the GMH of the RP.

At the U of C, we have changed our standard practice, as mice are no longer euthanized for RP regardless of size and no treatment is required. Mice identified with a RP are monitored at least twice a week due to the potential for necrosis and self-mutilation. However, necrosis or self-mutilation have not been observed at our institution since the beginning of this study in July 2019. While this study was intended to represent the general mouse population at a biomedical institution, other institutions may need to develop their own monitoring plan, as factors such as the microenvironment and type of research may influence the GMH and PAD. In adherence to the 3Rs, this study supports animal number reduction and clinical refinement, allowing mice to contribute to their intended research study endpoints or produce additional litters.

Acknowledgments

This project was funded by the University of Chicago, Animal Resources Center, Laboratory Animal Medicine Training Program. We thank Veterinary Technicians Chago Bowers, Karin Peterson, and Susan Zanelli for their time and participation in this study. We thank Dr. Jenna Schoenberger, for her time and assistance in this study. We thank Theodore Karrison, Research Professor and Director, Biostatistics Laboratory from the Department of Public Health Sciences, University of Chicago Biological Sciences for his time and skilled statistical support of this project. Finally, we thank Shari Hamilton for her guidance, work on this project and in creating the rectal prolapse histopathology scoring chart. This article was prepared while Dr. Salyards was employed at the University of Chicago. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

References

1. **Anderson DE, Miesner MD.** 2008. Rectal prolapse. Vet Clin North Am Food Anim Pract **24:**403–408. https://doi.org/10.1016/j.cvfa.2008.02.015.
2. **Barthold SW, Griffey SM, Percy DH.** 2016. Pathology of laboratory rodents and rabbits. Ames (IA): John Wiley and Sons. https://doi.org/10.1002/9781118924051
3. **Burkholder T, Foltz C, Karlsson E, Linton CG, Smith JM.** 2012. Health evaluation of experimental laboratory mice. Curr Protoc Mouse Biol **2:**145–165. https://doi.org/10.1002/9780470942390.mo110217.
4. **Cares K, Klein M, Thomas R, El-Baba M.** 2020. Rectal prolapse in children: an update to causes, clinical presentation, and management. J Pediatr Gastroentrerol Nutr **70:**243–246. https://doi.org/10.1097/MPG.0000000000002546.
5. **Chichlowski M, Hale LP.** 2009. Effects of Helicobacter infection on research: the case for eradication of Helicobacter from rodent research colonies. Comp Med **59:**10–17.
6. **Clark SJ.** 2016. Benign anal disease. JAAPA **29:**23–29.
7. **Crowder MJ, Hand DJ.** 1990. Analysis of repeated measures. Boca Raton (FL): Routledge. https://doi.org/10.1201/9781315137421
8. **Cunin D, Siproudhis L, Desfourneaux V, Berkelmans I, Meunier B, Bretagne J-F, Bouguen G.** 2013. No surgery for full-thickness rectal prolapse: what happens with continence? World J Surg **37:**1297–1302. https://doi.org/10.1007/s00268-013-1967-z.
9. **Czarnowicki T, Malajian D, Khattri S, da Rosa JC, Dutt R, Finney R, Dhingra N, Xiangyu P, Xu H, Estrada YD.** 2016. Petrolatum: barrier repair and antimicrobial responses underlying this "inert" moisturizer. J Allergy Clin Immunol **137:**1091–1102.e7. https://doi.org/10.1016/j.jaci.2015.08.013.
10. **Downard CD.** 2011. Perianal disease, p 461–465. In: Mattei P, editor. Fundamentals of pediatric surgery. New York (NY): Springer.
11. **Foltz C, Ullman-Cullere M.** 1998. Guidelines for assessing the health and condition of mice. Lab Anim (NY) **28:**28–32.
12. **Foster HL, Small JD, Fox JG.** 2014. The mouse in biomedical research: normative biology, immunology, and husbandry Burlington (MA): Academic Press.
13. **Fox JG, Anderson LC, Otto G, Pritchett-Corning KR, Whary MT.** 2015. Laboratory animal medicine. San Diego (CA): Elsevier Science. https://doi.org/10.1016/B978-0-12-409527-4.00001-8
14. **Gallo G, Martellucci J, Pellino G, Ghiselli R, Infantino A, Pucciani F, Trompetto M.** 2018. Consensus Statement of the Italian Society of Colorectal Surgery (SICCR): management and treatment of complete rectal prolapse. Tech Coloproctol **22:**919–931. https://doi.org/10.1007/s10151-018-1908-9.
15. **Haahr M.** [Internet]. 2019. True random number generator. [Cited 28 June 2019]. Available at: https://www.random.org.
16. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
17. **Kahn CM, Line S.** 2010. The Merck veterinary manual. Kenilworth (NJ): Merck.
18. **Kaplan EL, Meier P.** 1958. Nonparametric estimation from incomplete observations. J Am Stat Assoc **53:**457–481. https://doi.org/10.1080/01621459.1958.10501452.
19. **Kim J-k, Lee SH, Lee S-Y, Kim E-K, Kwon J-E, Seo H-B, Lee HH, Lee B-I, Park S-H, Cho M-L.** 2016. Grim19 attenuates DSS induced colitis in an animal model. PLoS One **11:**1–13. https://doi.org/10.1371/journal.pone.0155853.

20. **Kohn DF, Martin TE, Foley PL, Morris TH, Swindle MM, Vogler GA, Wixson SK.** 2007. Guidelines for the assessment and management of pain in rodents and rabbits. J Am Assoc Lab Anim Sci **46:**97–108.
21. **Mailhiot D, Ostdiek AM, Luchins KR, Bowers CJ, Theriault BR, Langan GP.** 2020. Comparing mouse health monitoring between soiled-bedding sentinel and exhaust air dust surveillance programs. J Am Assoc Lab Anim Sci **59:**58–66. https://doi.org/10.30802/AALAS-JAALAS-19-000061.
22. **Miller AL, Leach MC.** 2015. The mouse grimace scale: a clinically useful tool? PLoS One **10:**1–10. https://doi.org/10.1371/journal.pone.0136000.
23. **Miller CL, Muthupalani S, Shen Z, Fox JG.** 2014. Isolation of *Helicobacter* spp. from mice with rectal prolapses. Comp Med **64:**171–178.
24. **Mogil JS.** 2009. Animal models of pain: progress and challenges. Nat Rev Neurosci **10:**283–294. https://doi.org/10.1038/nrn2606.
25. **Mokhtar A, Abouheba M, Shehata S.** 2017. Evaluation of laparoscopic rectosigmoidopexy for the treatment of complete rectal prolapse in children. Minim Invasive Surg **1:**24–30. https://doi.org/10.20517/2574-1225.2016.09.
26. **Naydenov NG, Feygin A, Wang D, Kuemmerle JF, Harris G, Conti MA, Adelstein RS, Ivanov AI.** 2016. Nonmuscle myosin IIA regulates intestinal epithelial barrier in vivo and plays a protective role during experimental colitis. Sci Rep **6:**1–13. https://doi.org/10.1038/srep24161.
27. **Pettan-Brewer C, Treuting PM.** 2011. Practical pathology of aging mice. Pathobiol Aging Age Relat Dis **1:**1–16. https://doi.org/10.3402/pba.v1i0.7202.
28. **Pietroletti R.** 2018. Challenges in the surgical treatment of rectal prolapse. p 55–78. In: Proctological diseases in surgical practice. London (UK): British Library. doi: 10.5772/intechopen.78059
29. **Rao SS, Tetangco EP.** 2020. Anorectal disorders. J Clin Gastroenterol **54:**606–613. https://doi.org/10.1097/MCG.0000000000001348.
30. **Rogala AR, Morgan AP, Christensen AM, Gooch TJ, Bell TA, Miller DR, Godfrey VL, de Villena FP-M.** 2014. The collaborative cross as a resource for modeling human disease: CC011/Unc, a new mouse model for spontaneous colitis. Mamm Genome **25:**95–108. https://doi.org/10.1007/s00335-013-9499-2.
31. **Rose W, Zimmerman AC.** [Internet]. 1995. Petroleum jelly cream. U.S. Patent 5,407,678. [23 May 2019]. Available at: https://patents.google.com/patent/US5407678A/en
32. **Seamons A, Treuting PM, Brabb T, Maggio-Price L.** 2013. Characterization of dextran sodium sulfate-induced inflammation and colonic tumorigenesis in *Smad3*$^{-/-}$ mice with dysregulated TGFβ. PLoS One **8:**1–14. https://doi.org/10.1371/journal.pone.0079182.
33. **Turner PV, Pang DS, Lofgren JL.** 2019. A review of pain assessment methods in laboratory rodents. Comp Med **69:**451–467. https://doi.org/10.30802/AALAS-CM-19-000042.
34. **Uchihashi M, Wilding LA, Nowland MH.** 2015. Surgical correction of rectal prolapse in laboratory mice (*Mus musculus*). J Am Assoc Lab Anim Sci **54:**433–438.
35. **Ullman-Culleré MH, Foltz CJ.** 1999. Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Anim Sci **49:**319–323.
36. **Urban R, Scherrer G, Goulding EH, Tecott LH, Basbaum AI.** 2011. Behavioral indices of ongoing pain are largely unchanged in male mice with tissue or nerve injury-induced mechanical hypersensitivity. Pain **152:**990–1000. https://doi.org/10.1016/j.pain.2010.12.003.
37. **Varma M, Rafferty J, Buie WD.** 2011. Practice parameters for the management of rectal prolapse. Dis Colon Rectum **54:**1339–1346. https://doi.org/10.1097/DCR.0b013e3182310f75.
38. **Whary MT, Baumgarth N, Fox JG, Barthold SW.** 2015. Biology and diseases of mice, p 43–149. Chapter 3. In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT, editors. Laboratory animal medicine 3rd ed. Boston (MA): Academic Press.
39. **Woodruff K.** 2020. Rectal and vaginal fold prolapse, p 415–421. In: White S, editor. High-quality, high-volume spay and neuter and other shelter surgeries. Hoboken (NJ): John Wiley and Sons.
40. **Yiou R, Delmas V, Carmeliet P, Gherardi RK, Barlovatz-Meimon G, Chopin DK, Abbou C-C, Lefaucheur J-P.** 2001. The pathophysiology of pelvic floor disorders: evidence from a histomorphologic study of the perineum and a mouse model of rectal prolapse. J Anat **199:**599–607. https://doi.org/10.1046/j.1469-7580.2001.19950599.x.